Nicastrin Mutations in French Families with Hidradenitis Suppurativa

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TO THE EDITOR

Hidradenitis suppurativa (HS; OMIM 142690) is a chronic skin disease observed in 1% of the population and characterized by recurrent, deep abscesses located in the axillae, inguinal folds, and the buttocks. This affection is responsible for a very poor quality of life in the most severe patients. At least one-third of patients show an autosomal dominant inheritance (Fitzsimmons and Guilbert, 1985). Within the past few months, four reports involved γ-secretase mutations in familial HS (Wang *et al.*, 2010; Li *et al.*, 2011; Liu *et al.*, 2011; Pink *et al.*, 2011).

γ-Secretase is a transmembrane enzymatic complex that is composed of *Presenilin* (catalytic subunit), encoded by two homologs (*PSEN1* and *PSEN2*), and three cofactors: *Presenilin Enhancer 2* (*PSENEN*), *Nicastrin* (*NCSTN*), and *Anterior Pharynx Defective-1*, which also has two homologs (*APH1A* and *APH1B*). It catalyzes the cleavage of a wide range of transmembrane proteins, including β-amyloid precursor and Notch, which have generated a great interest because of their involvement in Alzheimer's disease and crucial developmental pathways, respectively (Shirotani *et al.*, 2004).

Herein we report the identification of *Nicastrin* mutations, which to our knowledge were not previously reported, in 3 out of 14 families of French origin with HS. A total of 104 individuals were recruited for this genetic study and signed a written informed consent. Diagnosis was confirmed according to Von Der Werth criteria (Von Der Werth *et al.*, 2000). The study complies with the principles of the Declaration of Helsinki Principles and was approved by the local ethics committee.

All affected members were non-obese and have been afflicted with HS since puberty. The patients of family 1 presented large hypertrophic scars and fistulas in the axillary, inguinal, and perianal folds with no spared area (Figures 2a and b). In families 2 and 3, the disease was less severe with intervals of normal skin in the same involved areas (Figures 2c–e). Associated diseases in affected members of these pedigrees included acne conglobata in pedigrees 1 and 2. Two affected members of pedigree 3 developed pelvispondylitis HLA B27(-), and one of them suffered from Crohn's disease.

Genomic DNA was extracted from venous blood or saliva. After having excluded *PSEN1*, *PSENEN*, *NCSTN*, and *APH1A* loci in two families by haplotype analysis, we proceeded to direct sequencing of the six γ-secretase genes in one affected individual from each of the remaining families (see Supplementary Text and Table S1 online). We also sequenced *PSEN2* and *APH1B* in the two excluded families.

In family 1, sequence analysis of γ-secretase genes revealed a nonsense mutation (c.1300C>T; GenBank accession NM_015331.2), which results in a premature termination codon in exon 11 of *NCSTN* (p.Arg434X; Figure 1a).

In the proband of family 2, we identified a heterozygous single-base deletion in exon 5 of *NCSTN* (c.487delC) leading to a frameshift and a premature termination codon (p.Gln163SerfsX39; Figure 1b).

In family 3, we found a heterozygous base-pair substitution (c.1768A>G) in exon 15 of *NCSTN* (Figure 1c). Interestingly, sequencing of cloned reverse-transcriptase–PCR products from peripheral blood mononuclear cells of one affected individual revealed that this A>G transition causes abnormal splicing, generating two aberrant transcripts in addition to the wild-type transcript arising from the nonmutated allele (Supplementary Figure S1 online). The first aberrant transcript resulted from the use of a cryptic splice-donor site located immediately downstream of the mutation leading to the deletion of the last 26 nucleotides of exon 15 and a premature termination codon (p.Ser590AlafsX3). The second aberrant transcript is spliced using the same cryptic splice-donor site in exon 15, and a newly activated cryptic splice-acceptor site within exon 16. This splicing event removed the last 26 nucleotides of exon 15 and the first 88 nucleotides of exon 16, resulting in an in-frame deletion of 114 nucleotides (Figure 2f). We found no evidence for normally spiced mutated allele.

Quantitative real-time reverse-transcriptase–PCR analysis showed a significant reduction of transcript levels of *NCSTN* in the patient of family 2 compared with controls, indicating that the mutant transcripts are subject to nonsense-mediated decay. In contrast, we did not observe reduction of *NCSTN* transcript levels in families 1 and 3 (data not shown).

In each family, these heterozygous mutations segregated with the disease phenotype and were not observed in 100 French control individuals. They were present neither in the dbSNP135 nor in the 1,000 genomes project.

Nicastrin is a glycoprotein acting as a γ-secretase complex stabilizer (Zhao *et al.*, 2010). It is, to date, the most frequently mutated gene among the γ-secretase subunits in familial HS cases. In this study, mutations in *NCSTN* were identified in 3 out of 14 pedigrees only, indicating the implication of other genes or the existence of causative mutations in noncoding regions of the known γ-secretase genes in these families.

The frameshift mutation in family 2 leads to haploinsufficiency, considering

Abbreviations: HS, hidradenitis suppurativa

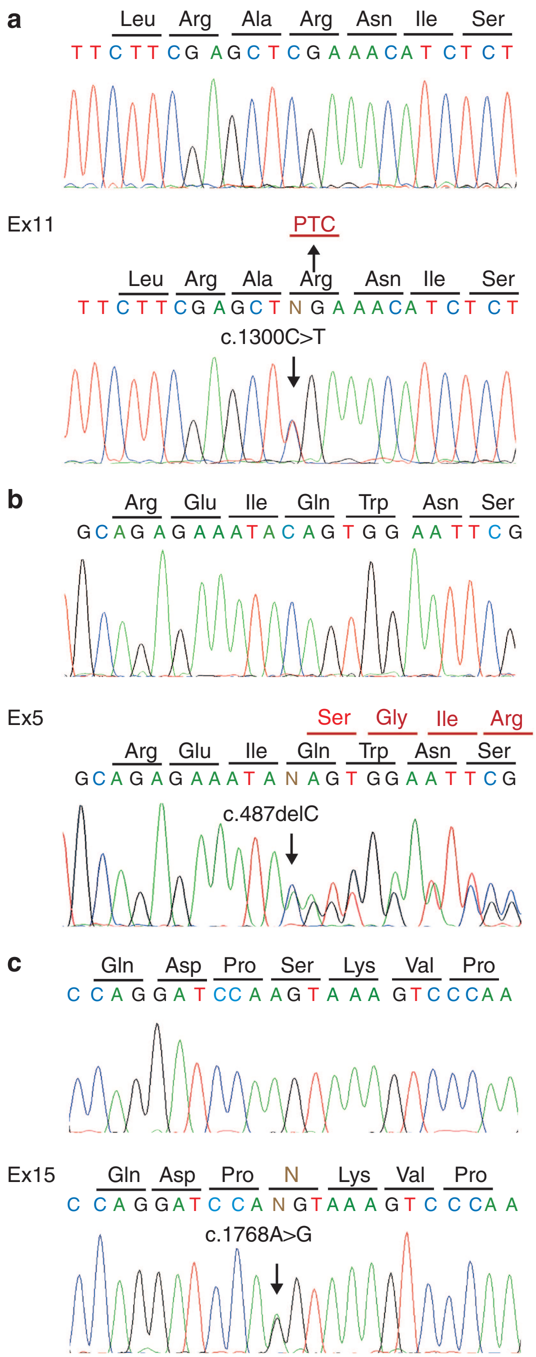

Figure 1. Sequencing chromatograms showing *NCSTN* mutations detected in the three French families affected with hidradenitis suppurativa. (**a**) Wild-type sequence and nonsense mutation (c.1300C > T; p.Arg434X) in pedigree 1. PTC, premature termination codon (TGA). (**b**) Wild-type sequence and single-nucleotide deletion (c.487delC; p.Gln163SerfsX39) in pedigree 2. Amino acids resulting from this frameshift mutation are highlighted in red. (**c**) Wild-type sequence and single-nucleotide substitution (c.1768A > G), which affects *NCSTN* pre-mRNA splicing in pedigree 3.

the severe reduction of *NCSTN* transcript levels in the patient. In families 1 and 3, the mutations may result in unstable proteins, leading to haploinsufficiency. It is also possible that the truncated protein in family 1 and the protein carrying the Δ26 deletion in family 3 are stable enough but fail to form the γ-secretase complex because they lack the transmembrane domain by which *Nicastrin* interacts with the other components of the complex (Capell *et al.*, 2003). Should the Δ114 mutated protein in family 3 be stable, we presume that it could fail in the correct folding required for the assembly and function of the γ-secretase complex (Shirotani *et al.*, 2003).

The pathophysiological mechanisms of HS remain unclear; however, several lines of evidence indicate that the Notch signaling pathway is likely to be involved. Indeed, Notch 1, 2, and 3 knockout mice develop a cutaneous phenotype that mimics the γ-secretase knockout phenotype, including cyst formation, and epidermal and hair follicle defects (Pan *et al.*, 2004). In addition, disruption of the nuclear target of Notch, *RBP-J*, also leads to epidermal cyst formation (Yamamoto *et al.*, 2003), a common hallmark in HS patients. These observations are consistent with a role of defective Notch signaling in HS and suggest that genes of the Notch pathway could be potential new candidate genes for this condition.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

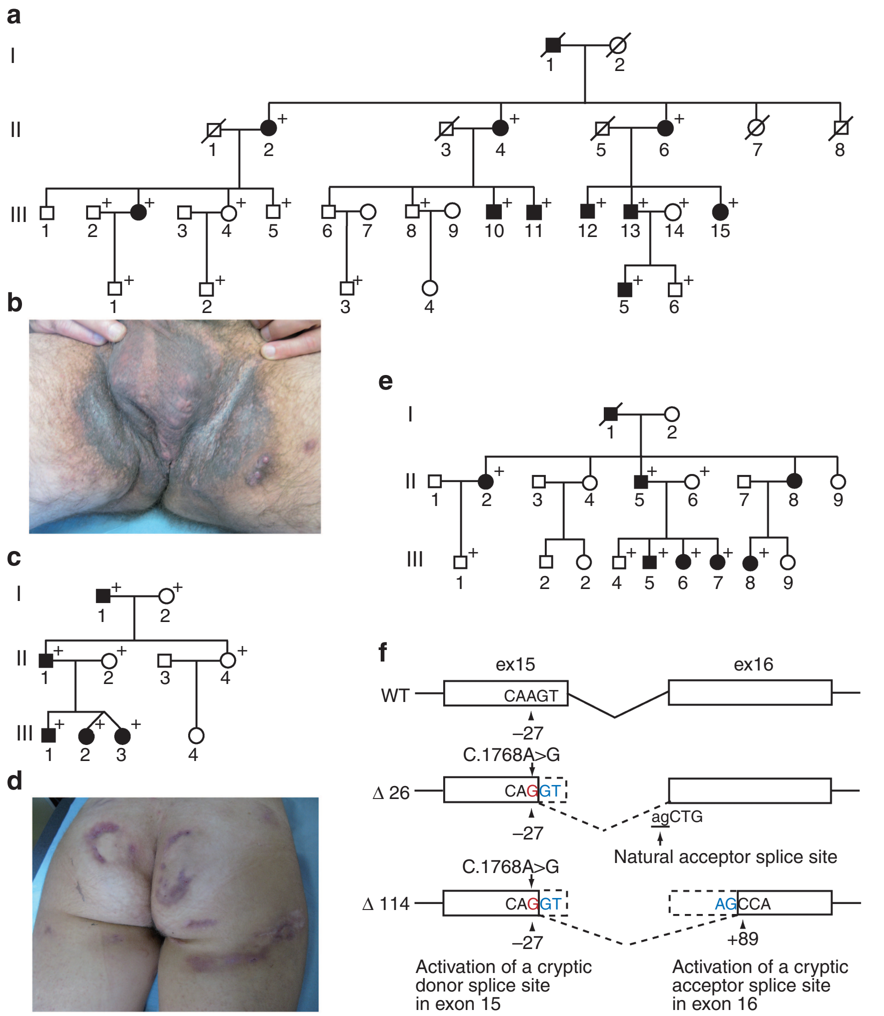

Figure 2. Genealogical trees, skin lesions in families 1 and 2, and differently spliced transcripts of *NCSTN* in family 3. "+" Indicates family members who were examined and sequenced. (**a**) Pedigree 1; (**b**) hypertrophic scars and nodules of the groin. (**c**) Pedigree 2; (**d**) hypertrophic scars and fistulas of the buttocks. (**e**) Pedigree 3; (**f**) three different transcripts were identified in the proband of family 3: a wild-type transcript (WT) and two transcripts derived from the c.1768A > G mutation: Δ26, resulting from a 26-bp deletion of exon 15 and Δ114, harboring a 26-bp deletion of exon 15 and an 88-bp deletion of exon 16. Newly activated acceptor (AG) and donor (GT) cryptic slice sites are highlighted in blue.

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Next-Generation DNA Re-Sequencing Identifies Common Variants of *TYR* and *HLA-A* that Modulate the Risk of Generalized Vitiligo via Antigen Presentation

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TO THE EDITOR

Generalized vitiligo (GV) is a common autoimmune disease resulting from the destruction of melanocytes in the involved areas, epidemiologically associated with elevated prevalence of certain other autoimmune diseases (Picardo and Taïeb, 2010). In a recent genome-wide association study (GWAS) of GV, carried out in European-derived whites (EUR), we identified 16 loci that contribute to GV risk (Jin *et al.*, 2010a,b; Birlea *et al.*, 2011).

Abbreviations: EUR, European-derived whites; GV, generalized vitiligo; GWAS, genome-wide association study; MHC, major histocompatibility complex; OCA1, oculocutaneous albinism type 1; OR, odds ratio; SNP, single-nucleotide polymorphism; TYR, tyrosinase (gene or protein)